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**Vibrational spectroscopy to predict in vitro digestibility and the maturity index of different forage crops during the growing cycle and after freeze- or oven-drying treatment**

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## UNIVERSITÀ DEGLI STUDI DI TORINO

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1    **NIRS instruments to predict *in vitro* digestibility and maturity index of different forage crops**  
2    **during the growing cycle and after oven drying or lyophilisation**

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## ABSTRACT

The aim of the study is to focus on the progression of intra- and inter-family maturity using near infrared spectroscopy (NIRS) on forage quality as a predictive tool. NIRS calibrations were developed on two instrument using two samples sets. The freeze-dried samples (n=158) and oven-dried samples (n=158) were examined using two NIRS instruments: a Perkin Elmer (PE, considering two bands: 714–2500 nm and 2501–3333 nm) and a portable Analytical Spectral Device (ASD, 350–1250 nm). NIRS technology was able to predict Daisy digestibility parameters more accurately using PE than with ASD for *in vitro* true digestibility over neutral detergent fiber digestibility. Using the PE instrument, the 2501–3333 nm band was 29% more efficient than the 714–2500 nm band. Digestible neutral detergent fiber content was fairly (ratio-performance deviation (RPD) 2.0 for PE) or not predicted (RPD 1.2 for ASD), while indigestible neutral detergent fiber was predicted by NIRS with accuracy (RPD 4.8 and 2.7, respectively). A multivariate maturity index based on composition, digestibility and tillage traits was formulated to rank species and botanic families according to their ontogeny, which was accurately predicted by the NIRS spectra (RPD 5.2 and 2.9 for PE and ASD, respectively).

**Keywords:** NIRS; Daisy *in vitro* digestibility; chemical composition; growth stage; chemometrics.

**Abbreviations:** ADFom, acid detergent fiber expressed exclusive of residual ash; ASD, analytical spectral device; CP, crude protein; D, days after seeding; DM, dry matter; dNDF, digestible neutral detergent fiber; EE, ether extract; GE, gross energy; iNDF, indigestible neutral detergent fiber; IVTD, *in vitro* true digestibility; MSE, mean square error; MI, maturity index; NDFD; *in vitro* neutral detergent fiber digestibility; NDFom, neutral detergent fiber expressed exclusive of residual ash; NIRS, near infrared spectroscopy; OM, organic matter; PE, perkin elmer; RPD, ratio-performance deviation; SD, standard deviation; standard error in cross-validation, SECV; VC=variation coefficient.

## 1. Introduction

Digestibility is the most common nutritive parameter used in feeding standards for ruminants (Coleman and Moore, 2003; NRC, 2001), the basal unit in evaluating the nutritive value of forage (Jancik et al., 2011; Wang et al., 2009). In fact, accurate estimation of forage digestibility is a prerequisite for diet formulation, economic evaluation of forages and prediction of animal responses (Ricci et al., 2009).

Digestibility can be estimated through several techniques, whose results can differ considerably (Huhtanen et al., 2006). Forage digestibility can be studied *in vivo*, *in situ* and *in vitro* (Cone et al., 1999). Chemical composition parameters have also been used to estimate the digestibility of forages, since it is well known that the structure and thus the components of the plant vary as the stage of maturity advances. However, the relationship between digestibility and chemical composition is very complex and depends on the botanical species (Bruinenberg et al., 2002). Moreover, digestibility prediction equations can be found in the literature, but despite their extensive use, evidence from existing studies suggests that their application to poor quality forages has been relatively unsatisfactory or inconsistent (Van Soest, 1994).

Given that *in vivo* determinations of digestibility are laborious, expensive and difficult to standardize, *in situ* and *in vitro* techniques have been developed (Gosselink et al., 2004; Stern et al., 1997). Much of this work has been done in ruminant species and has provided estimates highly correlated to *in vivo* digestibility values (Earing et al., 2010; Goldman et al., 1987; Stern et al., 1997).

Over the years, various procedures to determine digestibility have been developed and modified. Recently, an efficient alternative to the traditional *in vitro* method (Tilley and Terry, 1963) was developed by Ankom Technology (Fairport, NY, USA). This *in vitro* filter bag technique using Daisy<sup>II</sup> is a reliable and simple technique, which is easier and less time-consuming than the

conventional *in vitro* technique (Damiran et al., 2008; Holden, 1999; Mabjeesh et al., 2000; Truijillo et al., 2010). It has been shown to increase labor efficiency and precision. However, the technique involves *inoculum* of rumen fluid, which is the major factor introducing error into neutral detergent fiber digestibility assays (Goeser and Combs, 2009).

A possible alternative method could be the physical, rapid and non-destructive technique of near infrared spectroscopy (NIRS; Deaville et al., 2009). NIRS represents a radical departure from conventional analytical methods, in that the entire sample of a forage is characterized in terms of its absorption properties in the near infrared region. The technique offers advantages of simplicity, speed, no chemical waste and more cost-effective prediction. Even though it requires laborious calibration procedures and complexity in the choice of data treatment, NIRS has revolutionized the nutritional characterization of animal feed (Coleman and Moore, 2003; Givens and Deaville, 1999). NIRS has been shown to predict *in vivo* digestibility (Deaville et al., 2009; Decruyenaere et al., 2009; Landau et al., 2006).

The main objective of this study was to evaluate the potential of NIRS to predict *in vitro* digestibility assessed by the Daisy<sup>II</sup> system, adopting various different instruments and sample methods of preparation. A second aim of the study was to focus the inter-species and family variation into a maturity index for the crops, which would synthesize the information from all the available parameters, and that could also be correlated with near infrared spectra.

## **2. Materials and methods**

### *2.1. Plant material and chemical analyses*

Several sets of field data for borage (*Borago officinalis* L.; Peiretti et al., 2004), galega (*Galega officinalis* L.; Peiretti and Gai, 2006), false flax (*Camelina sativa* L.; Peiretti and Meineri, 2007), flax (*Linum usitatissimum* L.; Peiretti and Meineri, 2008), hemp (*Cannabis sativa* L.; Peiretti, 2009a), chia (*Salvia hispanica* L.; Peiretti and Gai, 2009), safflower (*Carthamus tinctorius* L.;

1 Peiretti, 2009b), sunflower (*Helianthus annuus* L.; Peiretti and Meineri, 2010), white lupin  
2 (*Lupinus albus* L.; Peiretti et al., 2010), perilla (*Perilla frutescens* L.; Peiretti, 2011), ravizzone  
3 (*Brassica campestris* L. var. *Oleifera*; Peiretti et al., 2012), and quinoa (*Chenopodium quinoa*  
4 Willd.; Peiretti et al., 2013), collected in various studies from 2002 to 2010, were used in this  
5 experiment. In total, 158 samples of these green crops were collected at progressive morphological  
6 stages, up to sub-maturity, to provide a large variability of quality parameters (Table 1).

7 Part of the fresh crop was chopped, frozen, freeze-dried, ground in a Cyclotec mill (Tecator,  
8 Herndon, VA, USA) to pass a 1mm screen, and then examined by NIRS.

9 A second aliquot of the whole plant samples was dried in a forced-draft oven at 60°C to  
10 constant weight to determine the dry matter (DM) content. Samples were then air-equilibrated,  
11 ground in a Cyclotec mill and stored for later analysis. Dried samples were analyzed to determine  
12 total N content according to the Dumas method, using a macro-N Nitrogen analyzer (Foss Heraeus  
13 Analysensysteme, Hanau, Germany), ash by ignition at 550°C, neutral detergent fiber (NDFom)  
14 without sodium sulfite and  $\alpha$ -amylase, and acid detergent fiber (ADFom) as described by Van Soest  
15 et al. (1991). Gross energy (GE) was determined using an adiabatic calorimeter bomb (IKA C7000,  
16 Staufen, Germany).

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## 18 2.2. *In vitro* digestibility and connected variables

19 Freeze-dried samples were analyzed to determine the *in vitro* true digestibility (IVTD) and *in*  
20 *vitro* neutral detergent fiber digestibility (NDFD) using the Daisy<sup>II</sup> Incubator (Ankom Technology  
21 Corp, Fairport, NY, USA) according to Robinson et al. (1999). The *in vitro* rumen incubations were  
22 performed in two consecutive fermentative runs. Ground samples (250 mg) were inserted into filter  
23 bags (Ankom F57 bags), which were then sealed. Digestion jars were filled with pre-warmed  
24 (39°C) buffer solutions (266 ml of solution A: KH<sub>2</sub>PO<sub>4</sub> 10 g/L, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.5 g/L, NaCl 0.5 g/L,  
25 CaCl<sub>2</sub>·2H<sub>2</sub>O 0.1 g/L, and urea 0.5 g/L; 1330 ml of solution B: Na<sub>2</sub>CO<sub>3</sub> 15.0 g/L, Na<sub>2</sub>S·9H<sub>2</sub>O 1.0

g/L) and placed into the incubator. Rumen liquor was collected from rumen contents obtained at a slaughterhouse from cattle (two run) of the same farm fed a fiber-rich diet (Peiretti et al., 2013). 400 ml of filtered liquor (through two layers of cheesecloth) was introduced into each jar together with the filter bags. After 48 h of incubation, bags were removed, rinsed thoroughly with cold tap water and immediately analyzed for NDFom content with the Ankom<sup>200</sup> Fiber Analyzer, following the Ankom Technology Method. Replicated analyses have been averaged by sample.

IVTD (g/kg DM) was calculated using the following equation:

$$1-(W_3-(W_1 \cdot C_1)) \cdot 1000 / (W_2 \cdot DM),$$

where  $W_1$  is the filter bag weight,  $W_2$  is the sample weight,  $W_3$  is the final weight (filter bag + residue) after *in vitro* and sequential treatment with neutral detergent solution,  $C_1$  is a comparison of the blank filter bag weight after and before digestion treatment and DM is the dry matter content of the samples.

NDFD (g/kg NDFom) was calculated using the following equation:

$$1-(W_3-(W_1 \cdot C_1)) \cdot 1000 / (W_2 \cdot NDFom)$$

where  $W_1$  is the filter bag weight,  $W_2$  is the sample weight,  $W_3$  is the final weight (filter bag + residue) after *in vitro* and sequential treatment with neutral detergent solution,  $C_1$  is a comparison of the blank filter bag weight after and before digestion treatment and NDFom is the neutral detergent fiber content of the sample.

The total neutral detergent fiber derives from the sum of the digestible neutral detergent fiber (dNDF, g/kg DM) and indigestible neutral detergent fiber (iNDF, g/kg DM), according to the neutral detergent fiber digestibility coefficient. The sum and the two *addendi* are statistically linked, then the neutral detergent fiber variance is:

$$\text{Var (NDF)} = \text{Var ( dNDF)} + \text{Var (iNDF)} + 2 \text{ Cov (dNDF, iNDF)}.$$

### 2.3. Statistical analyses



2.3.1. *Linear models.* A univariate GLM model (SAS/STAT® 9.2. SAS Inst. Inc., Cary, NC) was used to test the species (S), and the ontogenic factor was considered as days after seeding (D) on the digestibility traits, the connected variables and the chemical analyses:

$$Y_{ij} = M + S_i + R \cdot D_{ij} + E_{ij} \quad [1]$$

where: Y = variable of the  $i^{\text{th}}$  species of the  $j^{\text{th}}$  replicate; M = common average; S = effect of the  $i^{\text{th}}$  species; R = common regression effect of D;  $E_{ij}$  = error term.

The relevance of the covariate was quantified by a model with only the species:

$$Y_{ij} = M + S_i + E_{ij} \quad [2]$$

where: M = common average; S = effect of the  $i^{\text{th}}$  species;  $E_{ij}$  = error term.

The relevance of the regression factor was deducted as the difference between model [1] and model [2].

2.3.2. *Digestibility prediction from chemical constituents.* The study as to whether IVTD and NDFD can be predicted by chemical analyses were conducted without knowing the species and the phenological factors. The chemical equations were obtained by a stepwise regression of the predictand variable (Y) on all the predictor variables (X) provided of  $t > 2.0$ :

$$Y_m = K + \sum_{i=1}^6 \beta_i \cdot X_i + E_{ij} \quad [3]$$

where  $m = 1$ ; Y = IVTD;  $m = 2$ , Y2 = NDFD; K = constant;  $\beta_i = i^{\text{th}}$  regression coefficient for the  $i^{\text{th}}$  significant X variable.

2.3.3. *Maturity index.* A maturity index (MI) was formulated with a summative equation where the 8 variables were standardized, then weighted +1 if the regression of that variable (late) on D was positive and -1 if that variable (early) declined over time: in fact low (or negative) values, that indicate juvenile status, are opposed to more mature status for high (positive) maturity values. Lastly, the resulting sum was normalized to mean and standard deviation.

2.4. *Near infrared reflectance spectroscopy and chemometrics*

The FT-NIRS Spectrum IdentiCheck™ System (PE, Perkin-Elmer, Beaconsfield, England) was used to scan the freeze-dried and oven-dried samples in the range from 714 to 3333 nm. (2751 absorbance points). The LabSpec 4 Standard-Res Lab Analyzer portable fibre optic diode array spectrophotometer (ASD, Analytical Spectral Device Inc., Boulder, CO) was used to scan the same samples in the range from 350 to 1025 nm (676 absorbance points)

The native spectra of the two instruments were processed with mathematical pretreatment (standard normal deviate, de-trend, first derived, smoothed). The modified partial least squares method admitting one passage for elimination of outlier objects ( $t > 2.0$ ) and the cross-validation test were utilized to obtain the optimized equations for each considered variable. The prediction capacity of the calibrated models were then evaluated with the ratio-performance deviation (RPD; Williams and Sobering, 1996), a capacity parameter defined as the relationship between the standard deviation of the chemical method (SD reference) and the standard error in cross-validation (SECV) encountered in the NIRS model. When RPD values were  $\geq 2.5$ , relevant calibration models were considered to be suitable for routine use.

To study the relationships with the most relevant wavelengths for digestibility, chemical constituents and phenological variables, the FT-NIRS spectra from the PE instrument were segmented into two bands: Band-1 (714–2500 nm) and Band-2 (2501–3333 nm), a spectral band that is not often explored.

The RPD values were pooled over the variables and analyzed by linear models in order to differentiate the results pertaining to the combinations of the instrument, the spectral bands and the sample method used in preparing the specimen.

### 3. Results

#### 3.1. Characteristics of the species and phenological effects

Table 2 reports the characteristics of the twelve crops that were analyzed by the linear models.

The average r-square for species factor was 0.54, with the highest values for GE (0.87) and the lowest for crude protein (CP, 0.28). A wide range of significant differences spread the species across the variables with evidence for the extremes, i.e. flax, which reached maximum MI in DM at cutting, fiber fractions, GE and CP, with minimum digestibility parameters; at the opposite pole, borage showed minimal MI.

The r-square for the morphological stage accounted for an average 0.22 incidence (40% of the variability accounted for the species factor), varying from 0.04 for GE to 0.32 – 0.34 for the digestibility parameters. A negative significant regression on days after seeding (D) affected all of the digestibility parameters. As a sign of the ontogenic process, negative trends were exhibited by CP and ash, while DM at harvesting and all wall constituents increased substantially over time.

### 3.2. Maturity index

Table 3 reports the LsMeans for the MI of the crops and of the botanical families sorted by decreasing values. Several gradients of MI separated the twelve crops: flax (2.41 normal SD spread), false flax (2.01) and ravizzone (1.73) registered the highest mature values; hemp, galega, safflower, sunflower and white lupin were central; chia, perilla, quinoa, and borage represented the least mature type. When the twelve crops were grouped by family, five gradients divided their height families, namely from the most juvenile pole: *Boraginaceae* and *Chenopodiaceae* < *Lamiaceae* < *Asteraceae* and *Fabaceae* < *Cannabaceae* < *Brassicaceae* < *Linaceae* (most mature type).

### 3.3. Indirect prediction of digestibility traits and maturity index by chemical analyses

Few constituents were responsible for the digestibility parameters (Table 4). DM at cutting (negative sign) and ADFom (negative sign) were always present, while NDFom was relevant

(positive sign) for its own digestibility. The MI was perfectly predicted by all predictors, with positive contributions from GE, DM and ADFom.

### *3.4. Prediction of digestibility and chemical composition by NIRS*

The average absorbance spectra of the samples prepared by the oven-dried (O) or freeze-dried (F) methods, and scanned by PE or ASD instruments are reported in Fig. 1. Analogy between the instruments highlighted the oven-dried samples as being more absorbent of NIRS radiation than the freeze-dried preparations.

The highest NIRS performances (Table 5) were reached in the freeze-dried specimens with the PE instrument using Band-2 over 2500 nm (avg. RPD 4.31) followed by Band-1 (714–2500 nm), while the oven-dried preparations were much less reliable. By contrast, the sample preparation hardly affected ASD instrument performance (2.79 freeze-dried vs 2.45 oven-dried).

Among the predicted variables, the highest RPD ranking was achieved by MI (3.40) and the lowest (RPD < 2.5) by dNDF, CP and D.

When the spectra of the oven samples were merged with those of the freeze-dried samples in a data pool, the results of the NIRS predictions improved (Table 6) and the avg. RPD was 3.83 in the PE instrument vs 2.44 in the ASD instrument (+ 57%,  $P < 0.0005$ ) corresponding to avg. r-squares in cross-validation of 0.91 and 0.80, respectively. The highest RPD values were reached for maturity index, IVTD and the connected iNDF constituent, while the dNDF fraction was well predicted only by the PE instrument (RPD 2.0). Among the constituents, ash, NDFom and ADFom content obtained sound NIRS predictions.

Interesting features concerned two ontogenic traits: DM at harvesting was predicted with an error of  $\pm 1.4\%$  (RPD 3.3) and D with an error of  $\pm 4.4$  d (RPD 2.3) by the PE instrument.

## 4. Discussion

### 4.1. Domain of variation and co-variation for digestibility traits

NDFD is a predictor of forage digestibility that has been used for research purposes and routine forage analysis. Vendramini et al. (2010) noted a correlation between NDFD and IVTD in nine species and cultivars of warm-season grasses ( $r = 0.88$ ) similar to the 0.96 value in the present work. This very close relationship is due to the automatic correlation of a part with the whole. In effect, the predictions of the two traits based on chemical analysis pertain to the ADFom content and to the level of hydration at harvesting and to a lesser extent to the NDFom content.

Andres et al. (2005), in permanent meadow yield, reported a validated  $R^2$  of 0.82 for DM digestibility using the Goering and Van Soest (1970) methods, and observed that a summative equation from four laboratory constituents rose to  $R^2$  0.87, values similar to those for the Daisy IVTD estimates in the present work and based only on ADFom constituent and DM.

The results obtained by Smith et al. (1998) were more favorable for the direct estimation of digestibility in *Lolium rigidum* by NIRS technology ( $R^2$  0.94) than indirectly *via* chemical analyses. In this work, IVTD and NDFD were predicted respectively by chemical *vs* NIRS at  $R^2$  0.88 *vs* 0.94  $\div$  0.83 and 0.80 *vs*  $0.88 \div 0.75$ .

Similarly, Tran et al. (2010) on a consistent dataset of 2067 samples reported a 0.96  $R^2_{cv}$  for DM digestibility by NIRS of milking cow diets.

The relationship between digestibility and chemical composition is very complex and depends on the botanical species (Bruinenberg et al., 2002). Moreover, predictive equations for digestibility exist in the literature, but despite their extensive use, the evidence suggests that their application to poor quality forages has been relatively unsatisfactory or inconsistent between studies (Van Soest, 1994).

Nousiainen et al. (2004) in grass silage distinguished the digested part of neutral detergent fiber from iNDF; cross-validation statistics were higher for iNDF ( $R^2$  0.91) than for dNDF ( $R^2$

0.82); in that study, the standard deviations for NDFom, dNDF and iNDF were respectively 6/5/3%, while in our work they were 8/5/9%, highlighting higher amounts of indigestible fraction, due to a wider spectrum of ontological stages and botanical variability. Our results on IVTD agree with Mentink et al. (2006), who observed a validated  $R^2$  of 0.85 in total mixed rations, but was better than their 0.59 for NDFD.

Welle et al. (2003) investigated maize forage DM content and quality parameters by rapid NIRS directly on a harvesting machine. Results for enzymatic digestibility were poor ( $R^2$  0.39) but very reliable for starch (0.90), sugars (0.88) while for DM (0.96) they were even more reliable than laboratory tests.

#### *4.2. Near infrared spectra interpretation*

Clark and Lamb (1991) reviewed the selected wavelength to determine the DM digestibility of forages by NIRS. In a more complete framework of 9 reviewed works, having  $R^2_v$   $0.83 \pm 0.09$ , a pool of 57 reported nm values, two medians were featured:  $1700 \pm 50$  with 18% frequency and  $2300 \pm 50$  with 12% frequency. In the present work, the spectral region 1000–2500 nm privileged the 2278–2300 nm band for most of the traits (data not shown), while at 1756 nm only NDFD was correlated. Indeed, the traits were correlated with growing fit in the region above 2500 nm. The resulting poor resolution for all the variables in the first range of NIRS (714–1000) confirms Park et al. (1997) which in a comparison of three wavelength ranges (400–2500 nm; 700–2500 nm; 1100–2500 nm) on the effect of calibration performances for organic matter digestibility and intake observed little improvement on extending the range beyond 1100–2500 nm. By contrast, no reference is available about the remarkable advantage observed in the present work on extending the scan above 2500 nm.

#### *4.3. Maturity index proposal*

Attempts have been made to classify crops into high-, moderate- and low-quality crops, based on yield or nutritional value, mainly summarized in terms of digestibility (Bruinenberg et al., 2002). However, this separation into three categories of forage quality is arbitrary and still needs to take phenological evolution into account. In this paper, the incidence of the stage of maturity evolution (regression on D, Table 3) was evidenced by an r-square of 0.28 which was only half the value found for between-species variation (0.54); as a consequence, a large range of green and sub-mature crops, according to their ontogenic evolution, was exploited to build a robust calibration model. The proposed MI is adjusted for phenological footprint and has efficiently highlighted the botanical differences. The equations developed cannot aspire to field application, but since near infrared spectra have established reliable estimation capability, this synthetic approach coupled with this rapid tool, could be adapted to afford cheap and rapid genetic evaluation and improvement of grasses in complete agreement with the proposal by Humphreys (2005).

According to Mentink et al. (2006), NIRS is used in 60% of USA laboratories to predict feedstuff composition in the total mixed ration for dairy cows.

Several commercial testing laboratories offer wet chemistry NDFD measurements. NIRS calibrations for predicting NDFD on corn silage samples are available at some commercial forage testing laboratories. However, Lundberg et al. (2004) found poor prediction by NIRS of corn silage NDFD and hoped that NIRS calibration equations could be improved upon in the future.

In crops harvested from green to sub-mature stage, NIRS can potentially be used to predict Daisy digestion parameters with a precision that is almost equivalent to the prediction obtained by a regression based on any chemical parameters as predictors. Rapid knowledge of the digestibility parameter of total DM and especially of forage fiber will suggest the optimum point for cutting and, after harvesting, its rational use in ruminant feeding.

Several scientific opportunities (breeding, germplasm evaluation, technological and microbiological innovation, pre-selection of sub-samples from very large samples, etc.) justify the

existence of a rapid and cheap NIRS-based preliminary MI which could be progressively modulated and employed in the experimental decision process.

## References

- Andrés, S., Giraldez, F.J., Loéz, S., Mantecon, A.R., Calleja, S., 2005. Nutritive evaluation of herbage from permanent meadows by near-infrared spectroscopy: 1. Prediction of chemical composition and *in vitro* digestibility. J. Sci. Food Agric. 85, 1564–1571.
- Bruinenberg, M.H., Valk, H., Korevaar, H., Struik, P.C., 2002. Factors affecting digestibility of temperate forages from semi-natural grasslands: a review. Grass Forage Sci. 57, 292–301.
- Clark, D.H., Lamb, R.C., 1991. Near infrared reflectance spectroscopy: A survey of wavelength selection to determine dry matter digestibility. J. Dairy Sci. 74, 2200–2205.
- Coleman, S.W., Moore, J.E., 2003. Feed quality and animal performance. Field Crop. Res. 84, 17–29.
- Cone, J.W., Van Gelder, A.H., Soliman, I.A., De Visser, H., Van Vuuren, A.N., 1999. Different techniques to study rumen fermentation characteristics of maturing grass and grass silage. J. Dairy Sci. 82, 957–966.
- Damiran, D., Del Curto, T., Bohnert, D.W., Findholt, S.L., 2008. Comparison of techniques and grinding size to estimate digestibility of forage based ruminant diets. Anim. Feed Sci. Technol. 141, 15–35.
- Deaville, E.R., Humphries, D.J., Givens, D.I., 2009. Whole crop cereals 2. Prediction of apparent digestibility and energy value from *in vitro* digestion techniques and near infrared reflectance spectroscopy and of chemical composition by near infrared reflectance spectroscopy. Anim. Feed Sci. Technol. 149, 114–124.
- Decruyenaere, V., Lecomte, P., Demarquilly, C., Aufrere, J., Dardenne, P., Stilmant, D., Buldgen, A., 2009. Evaluation of green forage intake and digestibility in ruminants using near infrared



reflectance spectroscopy (NIRS): developing a global calibration. Anim. Feed Sci. Technol. 148, 138–156.

Earing, J.E., Cassill, B.D., Hayes, S.H., Vanzant, E.S., Lawrence, L.M., 2010. Comparison of *in vitro* digestibility estimates using the Daisy<sup>II</sup> incubator with *in vivo* digestibility estimates in horses. J. Anim. Sci. 88, 3954–3963.

Givens, D.I., Deaville, E.R., 1999. The current and future role of near infrared reflectance spectroscopy in animal nutrition: a review. Aust. J. Agr. Res. 50, 1131–1145.

Goering, H.K., Van Soest, P.J., 1970. Forage Fiber Analyses (Apparatus, Reagents, Procedures and Some Applications). USDA Handbook No 379. USDA, Washington, DC.

Goeser, J.P., Combs, D.K., 2009. An alternative method to assess 24-h ruminal *in vitro* neutral detergent fiber digestibility. J. Dairy Sci. 92, 3833–3841.

Goldman, A., Genizi, A., Yulzari, A., Seligman, N.G., 1987. Improving the reliability of the two-stage *in vitro* assay for ruminant feed digestibility by calibration against *in vivo* data from a wide range of sources. Anim. Feed Sci. Technol. 18, 233–245.

Gosselink, J.M.J., Dulphy, J.P., Poncet, C., Jailler, M., Tamminga, S., Cone, J.W., 2004. Prediction of forage digestibility in ruminants using *in situ* and *in vitro* techniques. Anim. Feed Sci. Technol. 115, 227–246.

Holden, L.A., 1999. Comparison of methods of *in vitro* dry matter digestibility for ten feeds. J. Dairy Sci. 82, 1791–1794.

Huhtanen, P., Ahvenjärvi, S., Weisbjerg, M.R., Nørgaard, P., 2006. Digestion and passage of fibre in ruminants. In: Sejrsen, K., Hvelplund, T., Nielsen, M.O. (Eds.), Ruminant physiology: Digestion metabolism and impact of nutrition on gene expression, immunology and stress, Wageningen Academic Publishers, Wageningen, The Netherlands, pp. 87–135.

Humphreys, M.O., 2005. Genetic improvement of forage crops - past, present and future. J. Agr. Sci. 143, 441–448.

1 Jancik, F., Rinne, M., Homolka, P., Cermak, B., Huhtanen, P., 2011. Comparison of methods for  
2 forage digestibility determination. *Anim. Feed Sci. Technol.* 169, 11–23.

3 Landau, S., Glasser, T., Dvash, L., 2006. Monitoring nutrition in small ruminants with the aid of  
4 near reflectance spectroscopy (NIRS) technology : a review. *Small Ruminant Res.* 61, 1–11.

5 Lundberg, K.M., Hoffman, P.C., Bauman, L.M., Berzaghi, P., 2004. Prediction of forage energy  
6 content by near infrared reflectance spectroscopy and summative equations. *Prof. Anim. Sci.*  
7 20, 262–269.

8 Mabjeesh, S.J., Cohen, M., Arieli, A., 2000. *In vitro* methods for measuring the dry matter  
9 digestibility of ruminant feedstuffs: comparison of methods and inoculum source. *J. Dairy*  
10 *Sci.* 83, 2289–2294.

11 Mentink, R.L., Hoffman, P.C., Bauman, L.M., 2006. Utility of near-infrared reflectance  
12 spectroscopy to predict nutrient composition and *in vitro* digestibility of total mixed rations. *J.*  
13 *Dairy Sci.* 89, 2320–2326.

14 Nousiainen, J., Ahvenjärvi, S., Rinne, M., Hellämäki, M., Huhtanen, P., 2004. Prediction of  
15 indigestible cell wall fraction of grass silage by near infrared reflectance spectroscopy. *Anim.*  
16 *Feed Sci. Technol.* 115, 295–311.

17 NRC, 2001. Nutrient requirements of dairy cattle, 7<sup>th</sup> ed. National Academy Press, Washington,  
18 DC, USA.

19 Park, R.S., Gordon, F.J., Agnew, R.E., Barnes, R.J., Steen, R.W.J., 1997. The use of near infrared  
20 spectroscopy on dried samples to predict biological parameters of grass silages. *Anim. Feed*  
21 *Sci. Technol.* 68, 235–246.

22 Peiretti, P.G., Palmegiano, G.B., Salamano, G., 2004. Quality and fatty acid content of borage  
23 (*Borago officinalis* L.) during the growth cycle. *Ital. J. Food Sci.* 2, 177–184.

1 Peiretti, P.G., Gai, F., 2006. Chemical composition, nutritive value, fatty acid and amino acid  
2 contents of *Galega officinalis* L. during its growth stage and in regrowth. Anim. Feed Sci.  
3 Technol. 130, 257–267.

4 Peiretti, P.G., Meineri, G., 2007. Fatty acids, chemical composition and organic matter digestibility  
5 of seeds and vegetative parts of false flax (*Camelina sativa* L.) after different lengths of  
6 growth. Anim. Feed Sci. Technol. 133, 341–350.

7 Peiretti, P.G., Meineri, G., 2008. Chemical composition, organic matter digestibility and fatty acid  
8 content of linseed (*Linum usitatissimum* L.) harvested at five stages of growth. J. Sci. Food  
9 Agric. 88, 1850–1854.

10 Peiretti, P.G., 2009a. Influence of the growth stage of hemp (*Cannabis sativa* L.) on fatty acid  
11 content, chemical composition and gross energy. Agric. J. 4, 27–31.

12 Peiretti, P.G., 2009b. Effects of growth stage on chemical composition, organic matter digestibility,  
13 gross energy and fatty acid content of safflower (*Carthamus tinctorius* L.). Livest. Res. Rural  
14 Develop. 21 (12), paper 206.

15 Peiretti, P.G., Gai, F., 2009. Fatty acid and nutritive quality of chia (*Salvia hispanica* L.) seeds and  
16 plant during growth. Anim. Feed Sci. Technol. 148, 267–275.

17 Peiretti, P.G., Meineri, G., 2010. Evolution of chemical composition, nutritive value, and fatty acid  
18 content of sunflower (*Helianthus annuus* L.) during the growth cycle. J. Anim. Vet. Adv. 9,  
19 112–117.

20 Peiretti, P.G., Daprà, F., Zunino, V., Meineri, G., 2010. The effect of harvest date on the chemical  
21 composition, gross energy, organic matter digestibility, nutritive value and amino acid content  
22 of white lupin (*Lupinus albus* L.). Cuban J. Agric. Sci. 44, 169–173.

23 Peiretti, P.G., 2011. Fatty acid content and chemical composition of vegetative parts of perilla  
24 (*Perilla frutescens* L.) after different growth lengths. Res. J. Med. Plant 5, 72–78.

1 Peiretti, P.G., Tassone, S., Gai, F., 2012. Nutritive quality and fatty acid profile of Ravizzone  
2 (*Brassica campestris* L. var. *Oleifera*) seeds and plant during growth. Livest. Res. Rural  
3 Develop. 24 (8), paper 142.

4 Peiretti, P.G., Gai, F., Tassone, S., 2013. Fatty acid and nutritive quality of quinoa (*Chenopodium*  
5 *quinoa* Willd.) seeds and plant during growth. Anim. Feed Sci. Technol. 183, 56–61.

6 Ricci, P., Romera, A.J., Burges, J.C., Fernandez, H.H., Cangiano, C.A., 2009. Case study. Precision  
7 and accuracy of methodologies for estimating *in vitro* digestibility of *Thinopyrum ponticum*  
8 (Tall wheatgrass) hay and haylage fed to beef cattle. Prof. Anim. Sci. 25, 625–632.

9 Robinson, P.H., Campbell, M., Fadel, J.G., 1999. Influence of storage time and temperature on *in*  
10 *vitro* digestion of neutral detergent fibre at 48 h, and comparison to 48 h *in sacco* neutral  
11 detergent fibre digestion. Anim. Feed Sci. Technol. 80, 257–266.

12 Smith, K.F., Simpson, R.J., Armstrong, R.D., 1998. Using near infrared reflectance spectroscopy to  
13 estimate the nutritive value of senescing annual ryegrass (*Lolium rigidum*): a comparison of  
14 calibration methods. Aust. J. Exp. Agric. 38, 45–54.

15 Stern, M.D., Bach, A., Calsamiglia, S., 1997. Alternative techniques for measuring nutrient  
16 digestion in ruminants. J. Anim. Sci. 75, 2256–2276.

17 Tilley, J.M.A., Terry, R.A., 1963. A two-stage technique for the *in vitro* digestion of forage crops.  
18 J. Brit. Grass. Soc. 18, 104–111.

19 Tran, H., Salgado, P., Tillard, E., Dardenne, P., Nguyen, T., Lecomte, P., 2010. “Global” and  
20 “local” predictions of dairy diet nutritional quality using near infrared reflectance  
21 spectroscopy. J. Dairy Sci. 93, 4961–4975.

22 Trujiillo, A.I., Marichal, M. de J., Carriquiry, M., 2010. Comparison of dry matter and neutral  
23 detergent fibre degradation of fibrous feedstuffs as determined with *in situ* and *in vitro*  
24 gravimetric procedures. Anim. Feed Sci. Technol. 161, 49–57.

1 Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent  
2 fiber, and non starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74, 3583–  
3 3591.

4 Van Soest, P.J., 1994. *Nutritional ecology of the ruminant*. (2nd ed.). Cornell University Press,  
5 Ithaca, New York.

6 Vendramini, J.M.B., Adesogan, A.T., Silveira, M.L.A., Sollenberger, L.E., Queiroz, O.C.,  
7 Anderson, W.E., 2010. Nutritive value and fermentation parameters of warm-season grass  
8 silage. *Prof. Anim. Sci.* 26, 193–200.

9 Wang, C.J., Tas, B.M., Glindemann, T., Rave, G., Schmidt, L., Weißbach, F., Susenbeth, A., 2009.  
10 Fecal crude protein content as an estimate for the digestibility of forage in grazing sheep.  
11 *Anim. Feed Sci. Technol.* 149, 199–208.

12 Welle, R., Gretenb, W., Rietmann, B., Alley, S., Sinnaeve, G., Dardenne, D., 2003. Near-infrared  
13 spectroscopy on chopper to measure maize forage quality parameters online. *Crop Sci.* 43,  
14 1407–1413.

15 Williams, P.C., Sobering, D., 1996. How do we do it: A brief summary of the methods we use in  
16 near infrared calibrations. In: Davies, A.M.C., Williams, P.C. (Eds.), *Near infrared*  
17 *spectroscopy: The future waves*, NIR Publications, Proceedings of the Seventh International  
18 Conference on Near Infrared Spectroscopy, Chichester, UK, pp. 185.